HEK-Blue™ hACE2 Cells

SEAP reporter HEK293 cells expressing human ACE2 gene

Catalog code: hkb-hace2

https://www.invivogen.com/hek-seap-hace2-cells

For research use only

Version 20K17-NJ

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 HEK-Blue™ hACE2 cells in a cryovial or shipping flask**

IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.

- **1 ml of Zeocin™** (100 mg/ml), store at 4 °C or at -20 °C.*
- **1 ml of Puromycin** (10 mg/ml), store at 4 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml): a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20°C.*

*The expiry date is specified on the product label.

- **1 pouch of HEK-Blue™ Detection**, a cell culture medium (50 ml) for real-time detection of SEAP. Store pouch at 4 °C for 6 months. Reconstituted HEK-Blue™ Detection is stable for 2 weeks at 4 °C. Protect from light.

Note: Data sheets for all components are available on our website.

Handling Frozen Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

Note: **Avoid freezing cells upon receipt** *as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

Cell Line Stability

Cells will undergo genotypic changes over time resulting in reduced responsiveness in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. HEK-Blue™ hACE2 cells should not be passaged more than 20 times to remain fully functional.

Quality Control

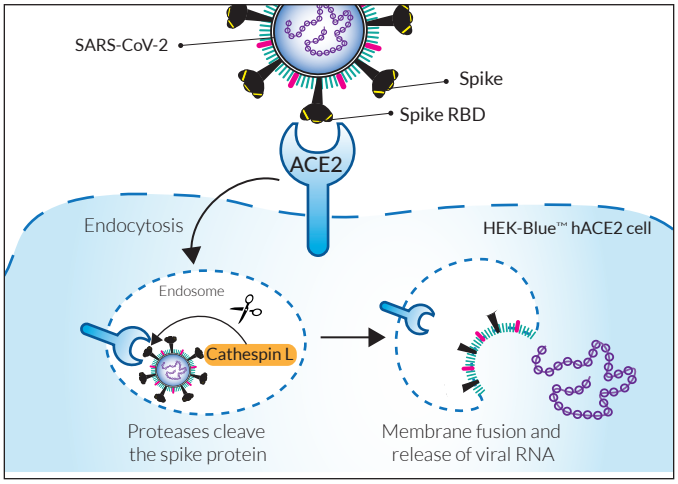
- The overexpression of the human *ACE2* (hACE2) gene has been verified by RT-qPCR, FACS staining, and functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

CELL LINE DESCRIPTION

HEK-Blue™ hACE2 cells were generated from HEK-Blue™ Null1-v cells, which derive from the human embryonic kidney (HEK)-293 cell line. HEK-Blue™ hACE2 cells overexpress the human ACE2 (hACE2) gene. Thus, unlike their parental cell line, they are permissive to infection with pseudotyped lentiviruses expressing the SARS-CoV-2 Spike protein. Additionally, they express a secreted embryonic alkaline phosphatase (SEAP) under the control of an NF-κB inducible promoter comprised of an IFN-β minimal promoter fused to five NF-κB and AP-1 binding sites. Levels of SEAP in the supernatant can be easily determined with HEK-Blue™ Detection, a SEAP detection cell culture medium. HEK-Blue™ hACE2 cells are resistant to Puromycin and Zeocin™.

BACKGROUND

ACE2 (angiotensin I-converting enzyme-2) is a type I membrane protein that belongs to the angiotensin-converting enzyme family[1]. It is expressed in arteries, heart, kidneys, and epithelia of the lung and small intestine[2]. Human ACE2 is the established host receptor for the Spike (S) protein of the SARS-CoV-2, the causative agent of COVID-19, enabling its entry into target cells[3-5]. In particular, SARS-CoV-2 gains entry to host cells through the binding of the Spike receptor-binding domain (RBD) to ACE2 at the cell surface[4,5]. Following this, host proteases, such as TMPRSS2 and Cathepsin L, allow the cleavage of the S protein into two subunits (S1 and S2), at the cell surface or in the endosomes, respectively. S2 mediates the fusion between the viral and host membranes and the viral contents are released into the cell[4-6]. Notably, TMPRSS2 is not needed for infection of HEK293 cells by SARS-CoV-2 spike-pseudotyped lentiviral particles[7].



1. Donoghue M. *et al.*, 2000. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Cir. Research. 87(5):e1-e9. **2. Harmer D. *et al.*, 2002.** Quantitative mRNA expression profiling of ACE2, a novel homolog of angiotensin-converting enzyme. FEBS Letters. 532(1-2):107-110. **3. Li W. *et al.*, 2003.** Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 426(6965):450-454. **4. Hoffmann M. *et al.*, 2020.** SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 181:1-16. **5. Zhou P. *et al.*, 2020.** A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 579(7798):270-273. **6. Walls A.C. *et al.*, 2020.** Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 181(2):281-292.e6. **7. Korber B. *et al.*, 2020.** Tracking changes in SARS-CoV-2-Spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell. DOI: 10.1016/j.cell.2020.06.043.

APPLICATIONS

HEK-Blue™ hACE2 cells are permissive to infection by SARS-CoV-2 and/or spike-pseudotyped lentiviral particles. Thus, they are ideal for studying viral entry into host cells, as well as for screening small molecule inhibitors and neutralizing antibodies. In addition, these cells express an NF-κB-inducible SEAP reporter and therefore, can be used to study how SARS-CoV-2 proteins interact and/or interfere with this signaling pathway (*see reporter assay*). Notably, despite the importance of TMPRSS2 for SARS-CoV-2 infection, it appears to be dispensible for viral entry into HEK293 cells expressing ACE2[7] (*and in-house data*).

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FAQ Any questions about our cell lines?
Visit our FAQ page.

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ hACE2 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require Biosafety Level 2 according to the American Center for Disease Control and Prevention (CDC) guidelines. The biosafety level may vary depending on the country. For example, in Germany HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 4.5 g/l glucose, 20% FBS, 10% DMSO

Note: Some FBS may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these thermosensitive enzymes.

- **Required Selection Antibiotics:** Puromycin and Zeocin™

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

Note: All of the steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells to a larger tube containing 15 ml of pre-warmed growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
4. Centrifuge tube at 200-300 x *g* for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6. Transfer the contents to a T-25 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the culture at 37°C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7x 10^6 cells/ml in freshly prepared freezing medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Dispense 1 ml of cell suspension into cryogenic vials.
3. Place vials in a freezing container and store at -80°C overnight.
4. Transfer vials to liquid nitrogen for long-term storage.

Note: If properly stored, cells should remain stable for years.

Cell maintenance

1. Maintain and subculture the cells in growth medium supplemented with 1 µg/ml of Puromycin and 100 µg/ml of Zeocin™.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: The hACE2 surface expression may be altered by the action of trypsin. We recommend you add pre-warmed phosphate buffered saline (PBS) and detach cells by tapping the flask.

Cell Handling Recommendations

To ensure the best results, use HEK-Blue™ hACE2 cells with less than 20 passages.

REPORTER ASSAY

We recommend to use HEK-Blue™ hACE2 cells with their corresponding parental cell line HEK-Blue™ Null1-v.

Note: For more information regarding the parental cell line please visit https://www.invivogen.com/hek-blue-null1v

1. Add 20 µl of each test sample per well of a 96-well flat-bottom plate. Include a positive control for both the parental and HEK-Blue™ hACE2 cells (i.e. recombinant human TNF-α), as well as a negative control (i.e. culture medium only).
2. Prepare a suspension of HEK-Blue™ hACE2 and their parental HEK-Blue™ Null1-v cells by gently rinsing the cells twice with pre-warmed phosphate buffered saline (PBS).
3. Add pre-warmed PBS (e.g. 2-5 ml for a T-75 flask) and incubate at 37°C for a few minutes.
4. Detach the cells by gently tapping the flask. Dissociate cell clumps by gently pipetting up and down.

Note: Do not use trypsin to detach HEK-Blue™ hACE2 cells.

5. Count the resuspended cells.
6. Prepare cell suspensions of ~280,000 cells per ml in HEK-Blue™ Detection medium and immediately add 180 µl of the cell suspensions (~50,000 cells) per well.

Note: Avoid prolonged incubation of cells at room temperature in HEK-Blue™ Detection medium as it may lead to high background or false positive readings.

7. Incubate the plate at 37°C in a CO_2 incubator for 16-24h. SEAP detection can be observed with the naked eye and accurately determined using a spectrophotometer at 620-655 nm.

Alternatively, SEAP can be detected using **QUANTI-Blue™ Solution**, a convenient and highly sensitive reagent that allows for repeated sampling or further experimentation. For more information please visit our website: https://www.invivogen.com/quanti-blue

USE RESTRICTIONS

These cells are distributed for research purposes only.

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RELATED PRODUCTS

Product	Cat. Code
Puromycin	ant-pr-1
Zeocin™	ant-zn-1
HEK-Blue™ Null1-v cells	hkb-null1v
pLV-SARS2-S-d19	plv-cov2-sd19
pLV-SARS2-S-d19 (D614G)	plv-cov2-sd19g
Spike-S1-Fc	fc-sars2-s1
Spike-S1-His	his-sars2-s1
Spike-RBD-Fc	fc-sars2-rbd
Spike-RBD-His	his-sars2-rbd
HEK-Blue™ Detection	hb-det2
QUANTI-Blue™ Solution	rep-qbs

HEK-Blue™ Detection

Cell culture medium for the real-time detection of secreted alkaline phosphatase

Catalog code: hb-det2, hb-det3

https://www.invivogen.com/hek-blue-detection

For research use only

Version 19F12-MM

PRODUCT INFORMATION

Contents

HEK-Blue™ Detection is provided in sealed pouches and is available in two quantities:

- hb-det2: 5 pouches
- hb-det3: 10 pouches

Each pouch contains everything needed to prepare 50 ml of medium for the colorimetric detection of secreted embryonic alkaline phosphatase (SEAP).

Storage and stability

- Store sealed pouches at 2-8 °C. Unopened pouches are stable for at least 6 months when stored properly.
Important: For the exact expiry date please see the corresponding CoA.
- Reconstituted HEK-Blue™ Detection is stable for 2 weeks at 2-8 °C and for 2 months at -20 °C. Protect from light.

DESCRIPTION

HEK-Blue™ Detection is a cell culture medium developed to provide a fast and convenient method to monitor SEAP expression. Detection of SEAP occurs as the reporter protein is secreted by the cells grown in HEK-Blue™ Detection, which will change to a purple/blue color in the presence of alkaline phosphatase activity.

SEAP is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a GPI-anchored protein. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters. It allows the determination of reporter activity without disturbing the cells, does not require the preparation of cell lysates, and can be used for kinetic studies. Using HEK-Blue™ Detection, SEAP expression can be observed visually, and unlike flourescent or luminescent reporters can be easily quantified using a microplate reader or spectrophotometer.

HEK-Blue™ Detection is applicable for high-throughput screening.

METHODS

Preparation of HEK-Blue™ Detection

1. Pour the contents of one pouch of HEK-Blue™ Detection into a sterile vial/bottle.
2. Solubilize the powder with 50 ml of endotoxin-free water.
3. Swirl gently until powder is completely dissolved.
4. Warm reconstituted HEK-Blue™ Detection to 37 °C for 30 minutes to 1 hour.
5. Filter the medium through a 0.2 µm membrane into a sterile vial/bottle.
6. Keep the HEK-Blue™ Detection medium at 37 °C before use or store at 2-8°C for up to 2 weeks.

Detection of SEAP activity

The following protocol is for the use of HEK-Blue™ Detection in 96-well plates. This will vary slightly depending on the volume of reagents needed, based on different plate sizes.

1. Prepare the cell suspension by detaching the cells and resuspending in a small volume of PBS.
2. Count the cells.
3. Add an appropriate amount of PBS-resuspended cells in HEK-Blue™ Detection to obtain a cell suspension at the expected concentration.
4. Add 20 µl of SEAP-inducer compound or negative control (such as PBS) per well.
5. Add 180 µl of cell suspension per well.
Note: To obtain more consistent results, we recommend to mix the SEAP-inducer and cell suspension by pipetting up and down.
6. Incubate overnight at 37 °C, in 5% CO_2.
7. Determine SEAP activity with the naked eye or by reading the optical density (OD) at 620-655 nm.

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (ZeoR)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
PlasmoTest™	rep-pt2
QUANTI-Blue™ Solution	rep-qbs

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